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Phase Transition and Segregation in Chromatin Reconstituted from Giant DNA

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長鎖 DNA (35 μm) とコアヒストンから再構成したクロマチンが、ヌクレオソーム密度に応じて急激な凝縮転移を起こし、ヌクレオソームの高密度な相と低密度な相に分離することを発見した。原子間力顕微鏡観察によりヌクレオソーム間の実効的相互作用ポテンシャルを求め、この凝縮転移を記述するモデルを提案した。

1 Introduction

Genomic DNA in eukaryotes is compactly folded into chromatin in the nucleus through several hierarchical packings. The unit of such packings is called nucleosome, in which DNA is wrapped 1.75 turns around protein called histone octamer. Since anionic DNA overcharges a cationic histone core, interaction between nucleosome is complicated. Although there have been many studies on the static and dynamic properties of nucleosomes, detailed mechanism on its condensation has not been clarified yet [2]. Here, we have studied the conformational change of individual reconstituted chromatin complex in order to shed light on the intrinsic property of “chromatin condensation.”

2 Materials and Methods

We performed the observation of reconstituted chromatin by fluorescence microscopy and atomic force microscopy (AFM). Chromatin was reconstituted from long plasmid DNA (contour length: 35 μm) and histone octamer, by the salt-dialysis method [3]. Based on a statistical analysis of the conformation, we interpret the free energy of a chromatin complex.

3 Results

Figure 1 (A–C) shows typical AFM images of reconstituted chromatin on a mica surface. The mass ratio $[\text{histone}]/[\text{DNA}]$ is 1.0 in (A) and 1.3 in (B) and (C). In picture (A), nucleosomes are dispersed in the chromatin, whereas in picture (B) the condensed and dispersed parts coexist.

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In picture (C) the chromatin is entirely condensed. It becomes clear that there is a large discrete transition between dense and dispersed states in reconstituted chromatin. Based on an analysis of the spatial distribution of the nucleosome cores, we deduced an effective interaction potential as $U(r) = -2.4r^{-9} + 12r^{-23}$, where r is the distance between nucleosomes normalized with the diameter of a nucleosome. Considering the apparent potential, thus obtained, together with elasticity of the DNA chain, we propose a hypothesis on the condensation of nucleosomes. Figure 1(D) shows the free-energy profile of chromatin with various numbers of nucleosomes n . With an increase in n , the free energy of the condensed state becomes the absolute minimum. It is found that chromatin undergoes a first-order phase transition.

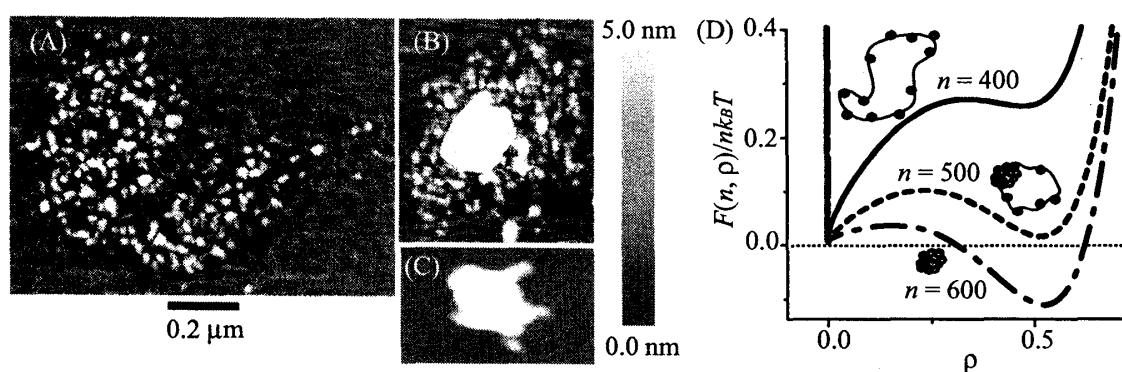


Figure 1: (A-C): AFM images of reconstituted chromatin. The mass ratio [histone]/[DNA] is 1.0 in (A) and 1.3 in (B, C). In picture (A), nucleosomes are dispersed in the chromatin, whereas in (B) the condensed and dispersed parts coexist. In (C) the chromatin is entirely condensed. (D): Free-energy profiles of a chromatin complex and corresponding schematic representations. n is the number of nucleosomes in a single chromatin complex and ρ is the normalized density of nucleosomes.

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